

**Total synthesis of Lavendamycin Analogs**

**An Honors Thesis (HONR 499)**

**By**

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I would like to dedicate my appreciation and gratitude to Dr. Robert Sammelson for giving me the opportunity to work on this project. His innovative mind, guidance, instruction and patience during the project truly inspire me to study chemistry, and will be invaluable to my future endeavors. I would also like to thank my parents, friends, and especially lab members for their help and support during this thesis.

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### Abstract

The substituents at the various positions on the quinone-5,8-dione determine its activation by NQO1 [NAD(P)H:quinone oxidoreductase 1], making the compound more toxic and specific towards NQO1-rich tumor cells. A series of reactions was conducted to synthesize the known 7N-acetamido-2-formylquinoline-5,8-dione. The aldehyde group of this quinolone-5,8-dione was transformed into the corresponding oxime. The oxime derivative was oxidized to the nitrile oxide in situ with NaOCl (bleach) and produced an isoxazoline or isoxazole through 1,3-dipolar cycloaddition with alkenes or alkynes, respectively. Recrystallization and column chromatography were performed to purify the products. The percent yields of the novel oxime, isoxazoline and isoxazole were 52%, 59%, and 71%, respectively. Further tests will be conducted to study the toxicity and affinity of the oxime, isoxazoline, and isoxazole for NQO1.

### Author's Statement

In the United States, cancer is the most common cause of death, exceeded only by heart disease, and accounts for nearly one of every four deaths. About 589,430 Americans were expected to die of cancer in 2015, or about 1,620 people per day<sup>2</sup>. The shocking figure does not stop there. It is estimated that approximately 1,658,370 new cancer cases were diagnosed in 2015<sup>2</sup>. This number does not include carcinoma in situ (noninvasive cancer) and basal cell skin cancers because they are not required to be reported to cancer registries.

Despite its detrimental effects, many people are not fully aware of cancer. Cancer is not a disease, but a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. If not treated, the spread can result in death. Cancer is caused by both external factors (such as tobacco, infectious organisms, and unhealthy diet) and internal factors (such as inherited genetic mutation, hormones, and immune conditions)<sup>2</sup>. While some cancers are curable, others require drugs to reduce symptoms and prolong survival. In 2004-2010, about 68% of cancer patients survived during the first five years of treatment, which is an improvement from 49% in 1975-1977<sup>2</sup>. New drugs and therapies are discovered every year to accommodate the ever complex and increasing growth of cancer. With the new and improved drugs, millions of lives can be saved. However, the dilemma is how to synthesize drugs that are selectively toxic toward tumor cells but leave normal cells intact.

Inspired to make a difference to the community as well as to test my chemistry lab skills, I challenged myself to work on the synthesis of lavendamycin derivatives in hope of finding a new and improved antitumor drug. The project proved to be long and challenging. Yet, I have learned so much from both Dr. Sammelson and my laboratory members. There were days when I could not get things to work and was very disappointed in myself. When a product was burned or

unable to purify, I had to either redo the reaction or change the direction of the research. Throughout the project, we had attempted multiple different approaches to synthesize quinolindione aldehyde efficiently as well as to test several solvent combinations for column chromatography. Through each failure, we learned from mistakes and made changes to the procedure to ensure the repeated experiment would be successful. As time passed, I realized the project was more difficult than what I initially thought. Somehow, the rewarding feeling that my project could potentially help millions of Americans combat against cancer kept me from giving up. Although I was only able to synthesize three new compounds, my contribution to the project would surely help other people to further the research on anticancer drugs.

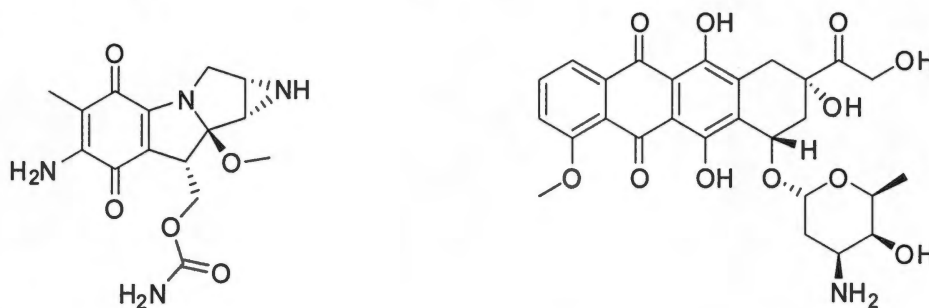
The research opportunity not only provides me a chance to positively impact my community, but also teaches me to incorporate my knowledge from other science classes into the research. The research itself is organic chemistry. However, the project could not be done without the help of other fields of science. In order for an anticancer drug to be marketed, it must pass biological studies. Thus, a sufficient knowledge in biochemistry, cellular biology and human physiology is necessary. Luckily, we were able to cooperate with University of Montana, who help us test the biological effects of the newly synthesized compounds.

By the time the project has come to an end, I realize the project itself is more than just a project. It is a delightful and enjoyable journey to gain knowledge, teamwork, and most importantly friendship with my laboratory members that will last forever. Hopefully, the newly synthesized compounds will work and more people will be saved from cancer.



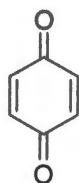
## Introduction

In the ongoing war against cancer, the goal of scientific research is to develop potent chemotherapy drugs that can selectively target tumor cells but are innocuous to normal cells.

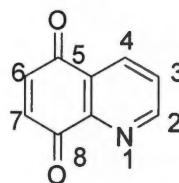


**Figure 1. Mitomycin C (A) and Adriamycin (Doxorubicin) (B)**

A few examples of chemotherapy drugs are Mitomycin C and Adriamycin (Doxorubicin). Both of these compounds act by blocking or stopping DNA replication and thus causing cell death<sup>8,13</sup>. Furthermore, they both possess a quinone structure. The quinone group undergoes oxidoreduction reactions to yield free radicals, mainly  $O_2^{\cdot -}$  and  $OH^{\cdot}$ , that are cytotoxic to tumor cells<sup>2</sup>.



**Figure 2. Quinone Structure**

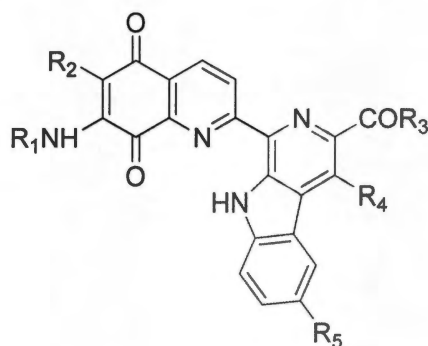


**Figure 3. Structure of quinoline-5,8-dione**

Inspired by these chemotherapy drugs, our research focuses on the synthesis of antitumor agents that also possess a quinone, or more specifically quinoline-5,8-dione, structure. Quinoline-5,8-dione is known for its antifungal, antibacterial, antiparasitic, and antitumor activity<sup>6</sup>. Quinoline-5,8-dione comes from the parent structure—lavendamycin.

Lavendamycin was first isolated from the fermentation broth of *Sreptomycetes lavendulae* by Balitz et al. in 1981<sup>3</sup>. Its structure, as illustrated by **Figure 4**, was determined by Doyle et al<sup>9</sup>.

Lavendamycin is pentacyclic, and consists of two moieties (a quinolone-5,8-dione, and a  $\beta$ -carboline). Despite its potent biological effects on tumors and bacteria, preclinical development of lavendamycin was halted due to its poor aqueous solubility and nonspecific cytotoxicity toward human cells<sup>5,7,10</sup>. In contrast to the parent compound, quinolone-5,8-dione has low animal toxicity and show strong antitumor activity as well as inhibition of HIV-reverse transcriptase<sup>10,11</sup>.

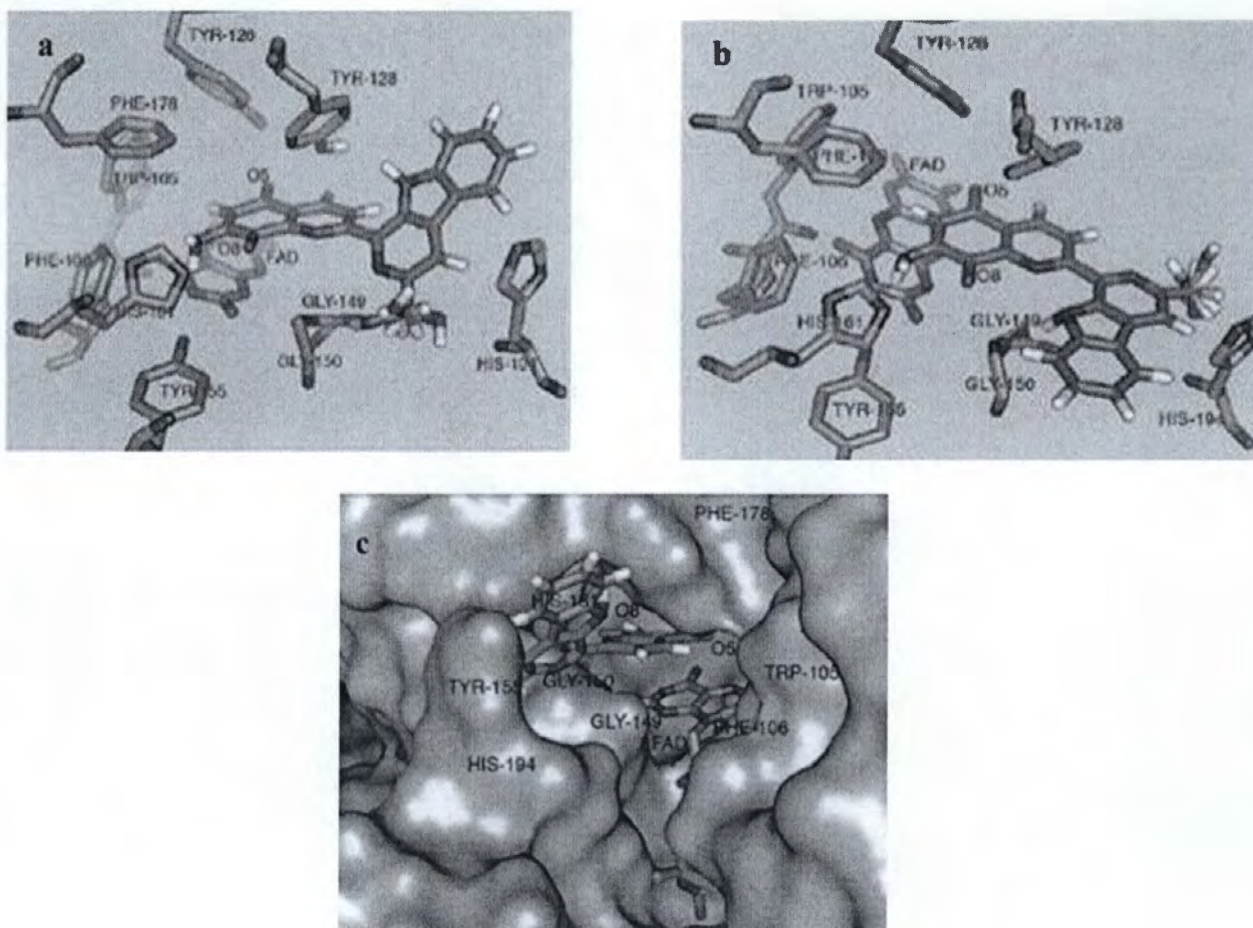


**Figure 4. Structure of lavendamycin with  $\beta$ -carboline shown in red**

At micromolar concentrations, lavendamycin strongly inhibits proliferation of P388 murine leukemia cells, MKN45 gastric carcinoma cells and WiDr colon carcinoma cells<sup>10</sup>. One study shows that lavendamycin is capable of attacking P-388J leukemia cells implanted into BDF1 mice<sup>10</sup>. Because of the lavendamycin's potent antitumor activity, researchers see a potential in developing anticancer drugs from its derivatives—something that is more suitable to the human body yet retains the potency of lavendamycin.

Biological studies of NQO1 activation by quinoline-5,8-dione have shown promising potentials for the development of antitumor agents that fit the research goal. NQO1 stands for NAD(P)H:quinone oxidoreductase 1. This enzyme is expressed at high levels in cancer cells, and capable of catalyzing quinone-based compounds<sup>11</sup>. At the NQO1 active site, quinone substrates bind in different orientations. **Figure 5a** and **5b** show two ways a lavendamycin analogue can bind to the FAD cofactor molecule present at the NQO1 active site. **Figure 5c** shows the

molecular surface of the enzyme active site, which is a hydrophobic and plastic pocket with three potential hydrogen-bonding residues<sup>11</sup>. Once activation occurs, the quinolinedione undergoes two electron reduction (known as the ping-pong mechanism) and activates cell death. Although the exact cause of apoptosis is unknown, research conjectures that it could be due to depletion of NADPH/NADH, coupling of oxidative phosphorylation, and/or single-strand cleavage of DNA<sup>12</sup>.



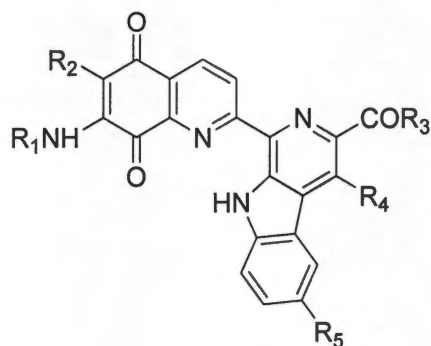
**Figure 5. The Binding Site of NQO1 Enzyme<sup>11</sup>**

The trick to synthesizing effective quinolone-5,8-dione is to install appropriate groups that can interact properly to the human cells. Previously, the binding of lavendamycin to the cell's enzyme is done via the  $\beta$ -carboline attached to C-1 of quinoline-5,8-dione. Our research

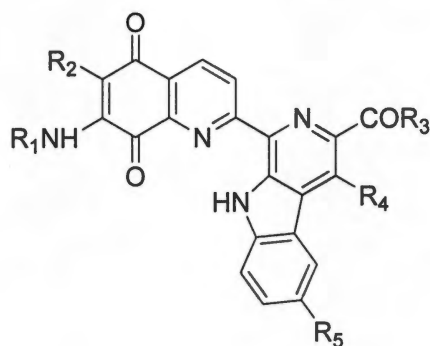


goal is to create a new group at the C-1 position that fits the enzyme's active site better, thus amplifying the antitumor activity of the compound. Previously, research has focused on the chemistry and/or bioassays effects of R groups at position 6 and 7 of the quinolone-5,8-dione. The substituents mainly include alkyl, halogen, nitro groups, amino, hydroxyl, thiol and their derivatives<sup>6</sup>. Research by Behforouz et al. suggests that the small the R groups at C-6 and C-7, the higher the selectivity and metabolism rate by NQO1<sup>6</sup> (**Table 1 and 2**). This study was conducted to synthesize and test biological effects of different R groups at C-1 while keeping the R groups at C-6 and C-7 the same (hydrogen and acetamido, respectively). In addition, the study aims to optimize previous procedure as well as to increase the overall yield of the quinolinedione aldehyde, which is an intermediate molecule of the whole process.

Table 1. The Effects of R groups on Metabolism<sup>6</sup>



Entry	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	Metabolism ( $\mu\text{mol/min/mg}$ )
1	CH <sub>3</sub> CO	H	N[(CH <sub>2</sub> ) <sub>4</sub> ]	H	H	43 $\pm$ 7
2	CH <sub>3</sub> CO	H	NH(CH <sub>2</sub> ) <sub>2</sub> OH	H	H	2.2 $\pm$ 0.7
3	CH <sub>3</sub> CO	H	OCH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>	H	H	3.9 $\pm$ 2.0
4	CH <sub>3</sub> CO	H	NHCH <sub>2</sub> CH(OH)CH <sub>2</sub> OH	H	H	36 $\pm$ 6
5	CH <sub>3</sub> CO	H	O(CH <sub>2</sub> )CH <sub>3</sub>	H	H	6.8 $\pm$ 3.5
6	CH <sub>3</sub> CO	H	N[(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> ]NCH <sub>2</sub> Ph	H	H	8.7 $\pm$ 1.5
7	CH <sub>3</sub> CO	H	OCH <sub>2</sub> CH <sub>3</sub>	H	H	3.6 $\pm$ 0.3
8	CH <sub>3</sub> CO	H	OCH <sub>2</sub> CH <sub>3</sub>	H	OH	21 $\pm$ 6
9	(CH <sub>3</sub> ) <sub>2</sub> CHCO	H	OCH <sub>3</sub>	H	H	0.04 $\pm$ 0.08
10	HCO	H	OCH <sub>3</sub>	H	H	20 $\pm$ 8
11	ClCH <sub>2</sub> CO	H	NH <sub>2</sub>	H	H	13 $\pm$ 2
12	ClCH <sub>2</sub> CO	H	N[(CH <sub>2</sub> ) <sub>4</sub> ]	H	H	34 $\pm$ 5
13	2-Furyl-CO	H	OCH <sub>3</sub>	CH <sub>3</sub>	H	5.8 $\pm$ 1.7
14	HO <sub>2</sub> C(CH <sub>2</sub> ) <sub>2</sub> CO	H	OC <sub>4</sub> H <sub>9-n</sub>	H	H	5.2 $\pm$ 2.6
15	H	Cl	OCH <sub>2</sub> CH <sub>3</sub>	H	H	6.9 $\pm$ 1.1
16	H	H	N[(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> ]O	H	H	103 $\pm$ 8
17	H	H	O(CH <sub>2</sub> ) <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	H	OH	2.9 $\pm$ 1.4
18	H	H	OCH <sub>3</sub>	CH <sub>3</sub>	OH	31 $\pm$ 7
19	H	H	N[(CH <sub>2</sub> ) <sub>4</sub> ]	H	H	35 $\pm$ 7
20	H	H	NH(CH <sub>2</sub> ) <sub>2</sub> OH	H	H	26 $\pm$ 4
21	H	H	OCH <sub>3</sub>	H	H	28 $\pm$ 2

Table 2. The Effects of R groups on Selectivity<sup>6</sup>


Entry	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>	Selectivity ratio BE/BE- NQ
1	CH <sub>3</sub> CO	H	N[(CH <sub>2</sub> ) <sub>4</sub> ]	H	H	2.1
2	CH <sub>3</sub> CO	H	OCH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>	H	H	0.8
3	CH <sub>3</sub> CO	H	NHCH <sub>2</sub> CH(OH)CH <sub>2</sub> OH	H	H	2.8
4	CH <sub>3</sub> CO	H	O(CH <sub>2</sub> )CH <sub>3</sub>	H	H	1.3
5	CH <sub>3</sub> CO	H	N[(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> ]NCH <sub>2</sub> Ph	H	H	0.7
6	CH <sub>3</sub> CO	H	OCH <sub>2</sub> CH <sub>3</sub>	H	H	1.3
7	CH <sub>3</sub> CO	H	OCH <sub>2</sub> CH <sub>3</sub>	H	OH	0.8
9	HCO	H	OCH <sub>3</sub>	H	H	1.4
10	ClCH <sub>2</sub> CO	H	NH <sub>2</sub>	H	H	2.1
11	ClCH <sub>2</sub> CO	H	N[(CH <sub>2</sub> ) <sub>4</sub> ]	H	H	2.7
12	HO <sub>2</sub> C(CH <sub>2</sub> ) <sub>2</sub> CO	H	OC <sub>4</sub> H <sub>9-n</sub>	H	H	1.6
13	H	H	N[(CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> ]O	H	H	9.0
14	H	H	OCH <sub>3</sub>	CH <sub>3</sub>	OH	2.5
15	H	H	N[(CH <sub>2</sub> ) <sub>4</sub> ]	H	H	1.1
16	H	H	NH(CH <sub>2</sub> ) <sub>2</sub> OH	H	H	1.0
17	H	H	OCH <sub>3</sub>	H	H	2.0

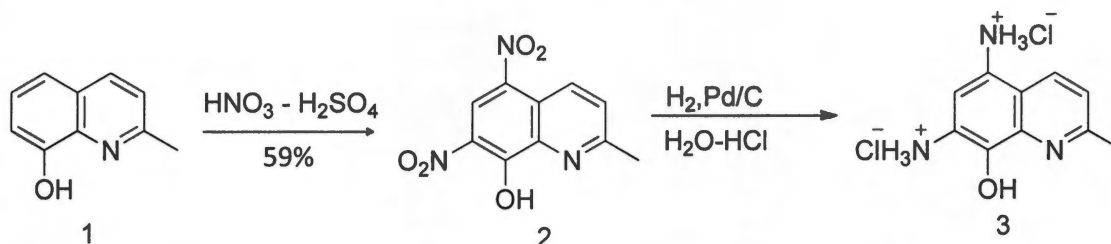
In our research, isoxazole and isoxazoline are chosen to be the new substituent at C-1 while the  $R_1$  group is kept as acyl. Pharmaceutical research has shown that isoxazole and isoxazoline derivatives display anticonvulsant, antimicrobial, and antitumor activity<sup>1,4</sup>. In addition, they play a crucial role as intermediates in the organic synthesis of many heterocyclic pharmacological active compounds<sup>1</sup>. Already marketed drugs like Valdecoxib as COX-2 inhibitor, Cloxacillin and Dicloxacillin as beta lactamase resistant antibiotics, which have isoxazole core, have been proven to be effective in respective disease scenario<sup>4</sup>. Preliminary evaluation of several isoxazole derivatives demonstrates that they are excellent anticancer agents<sup>14</sup>. The synthesis and biological study on isoxazole and isoxazoline substituted quinoline-5,8-dione will give valuable insights into what functional groups can enhance the potency of the compound.



**Figure 5. Isoxazole (A) and Isoxazoline (B)**



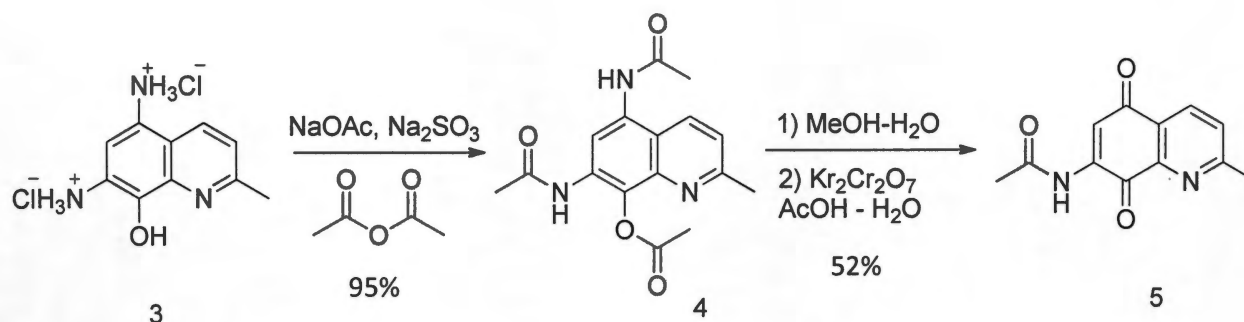
# Results and Discussion



**Scheme 1. Synthesis of 5,7-diamino-8-hydroxy-2-methylquinoline**

In the first step, 8-hydroxy-2-methyl-5,7-dinitroquinoline (2) was produced from commercially available 8-hydroxyquinoline. The product displayed two colors (green and yellow). Yet the  $^1\text{H}$  NMR spectra of both samples showed the same peaks. A possible explanation for this phenomenon is the difference in reflection and absorption of light from both the inner and outer surface of the compound.

When preparing 5,7-diamino-8-hydroxy-2-methylquinoline dihydrochloride (3), the product was not rotovapped to dryness, but kept as a solution for the next step. In previous procedure, water was supposedly added in the next step. Thus, skipping over the rotovaporation step would save time and eliminate the chance of contamination from rotary evaporator. The amount of water used to wash 5,7-diamino-8-hydroxy-2-methylquinoline dihydrochloride (3) must be kept at minimal. Too much water seemed to intervene with the next reaction and caused lower yield. Sometimes, the product was rotovapped to reduce the volume of water rather than to get rid of all solvents.



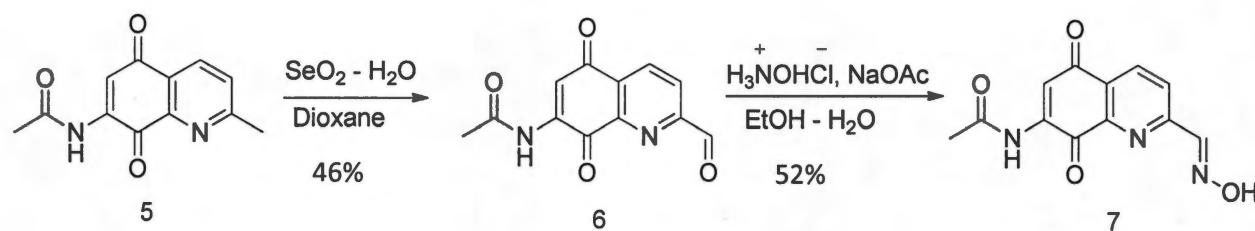
**Scheme 2. Synthesis of 7N-acetamido-2methylquinoline-5,8-dione**

The reaction of 5,7N-Diacetamide-8-acetoxy-2-methylquinoline (4) often yielded over 100%. The product must be allowed to dry for a day. Acetic anhydride should be added slowly for precipitate to form properly. Rushing over this step might result in lower yield. Several modifications were made to the preparation of 7N-acetamido-2-methylquinoline-5,8-dione (5) to decrease reaction time as well as to increase the percent yield. In this step, both the amount water and acetic acid were modified from previous procedure in hope of reducing reaction time. Water was used to convert potassium dichromate to chromic acid. When the amount of water was reduced from 43mL to 11mL, the percent yield was almost the same. This could either mean the product was not completely extracted from the aqueous layer or the amount of water did not play a major role in the reaction.

With little water, it was impossible to distinguish the layers. 50 mL of sodium bicarbonate was added to create an aqueous layer. Unlike water, when acetic acid was reduced from 45 mL to 21 mL for 3.17 mmol of 5,7N-diacetamide-8-acetoxy-2-methylquinoline (4), no acid was found in the organic layer. Previous, having too much acid usually caused the organic layer to have a red/orange color, even upon vigorous shaking. The only way to get rid of the red color was to let the solution sit in the reparatory funnel until the color turned clear yellow

(approximately one week). New sodium bicarbonate was added occasionally to make the red color fade faster. Without changing the sodium bicarbonate solution, it would take two weeks for the change in color to occur. Even when acid was reduced, solution was left in the separatory funnel for an extra night to ensure the removal of all acid.

In addition, repeated experiments demonstrated that efficient yield could not be achieved on larger scales. The smaller the scale is, the higher the yield. Since mass production of 7N-acetamido-2-methylquinoline-5,8-dione (5) was necessary to make sufficient amount of isoxazoline and isoxazole, 3-5 mmol of 5,7N-diacetamide-8-acetoxy-2-methylquinoline (4) should be used in this reaction. Percent yield dropped significantly when 10 mmol was used.

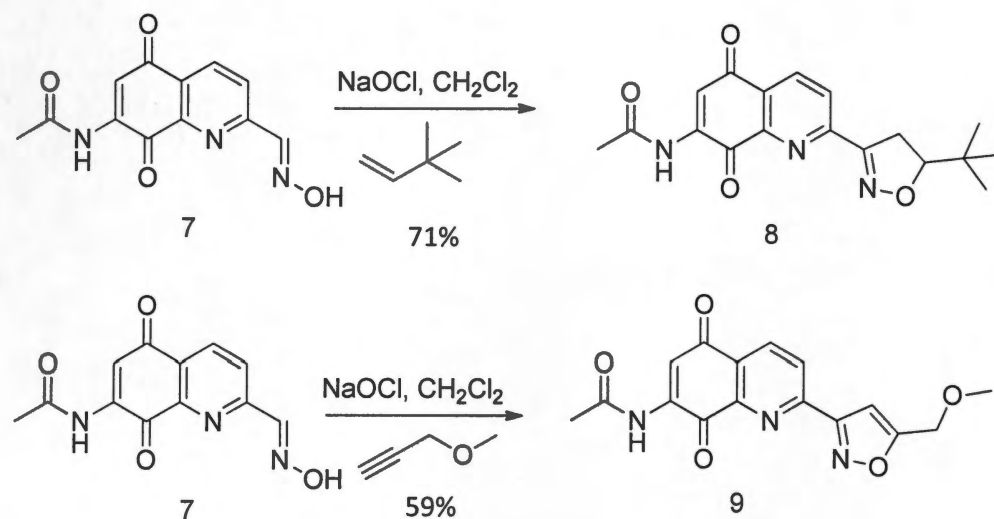


**Scheme 3. Synthesis of oxime**

7N-Acetamido-2-formylquinoline-5,8-dione (6) proved to be the most challenge step. Many procedures called for a 2 or 3-necked round bottomed flask. Although having extra necks made it easier to add selenium and water during the reflux, they were unnecessary. The solution vapor could easily get trapped in the rubber stoppers, leading to low yield and contamination. In addition, this reaction should not be done on a large scale due to high impurities and low yield. The ratio of selenium dioxide to 7N-acetamido-2-methylquinoline-5,8-dione (5) should be 1.2~3 to 1. When the ratio was over 2:1, product had a brown/red color which was indicative of impurities.

Previous procedure called for Celite cake to get rid of selenium. However, it was inefficient in our study since the product seemed to be trapped in the Celite cake and had an orange/brown color at the end. Column was attempted, but was ineffective. The majority of the product was lost in the column. To replace the Celite cake, a Celite column made with a glass pipette and a cotton ball. Because the column might not be packed tightly, air was not advised to use. The organic layer was washed with sodium chloride and sodium bicarbonate to get rid of extra selenium and acid. Drying the organic layer with magnesium sulfate might be skipped if high vac was used. This change in procedure avoided the loss of the product from transferring between glassware.

Oxime (7) had a very poor organic solubility. When recrystallizing with ethyl acetate, the product was not completely dissolved. Thus, purification was not achieved and column chromatography was attempted. Due to the poor recovery from column, using clean starting materials was the best way to achieve pure product.



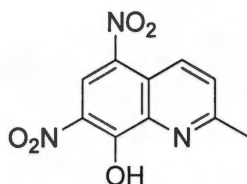
**Scheme 4. Synthesis of isoxazoline and isoxazole**



Isoxazoline (8) and isoxazole (9) were not as tricky as the aldehyde (6) substituent. The first few drops of bleach were added very slowly to ensure the reaction occurs favorably. Oftentimes, the process took more than 30 minutes (usually 1 hour on average). Vigorous stirring was necessary. Weak stirring resulted in lower yield and the color remained yellowish green. Good isoxazole and isoxazoline should have bright yellow color. Because recrystallization did not seem to work, column chromatography was the only way to purify the product, which resulted in lower yield.

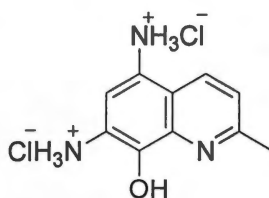
## Experimental

### Preparation of 8-Hydroxy-2-methyl-5,7-dinitroquinoline (2)



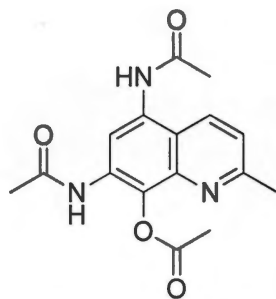
In a 125 mL Erlenmeyer flask cooled by ice-water bath, 3 mL of sulfuric acid and 2.0 g (12.3 mmol) of 8-hydroxyquinoline were combined and stirred. Ice cold concentrated nitric acid (5 mL) was added dropwise over 30 minutes. The solution was brought to room temperature and stirred for an additional one hour. The dark brown solution was added to 100 mL of ice/water and the bright yellow precipitate was collected via vacuum filtration. The solid was washed with water and dried overnight to yield 3.07 g (59%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  2.95 (s, 3H), 8.15 (d,  $J$  = 8.9 Hz, 1H), 9.21 (s, 1H), 9.67 (d,  $J$  = 8.9 Hz, 1H).

### Preparation of 5,7-Diamino-8-hydroxy-2-methylquinoline dihydrochloride (3)



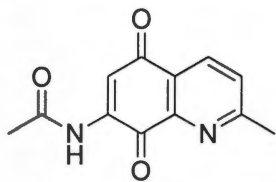
In a 500mL Parr hydrogenation bottle, 8-hydroxy-2-methyl-5,7-dinitroquinoline (9.0 g, 6.1 mmol) and 5% Pd/C (2.0 g, 0.9 mmol) were added to a solution made with 15 mL of 12M HCl and 135 mL of water. The mixture was shaken on the hydrogenator for 18h at 38psi. The dark red solution was vacuum filtered and washed with distilled water. The filtrate was kept for the next step.

### Preparation of 5,7N-Diacetamide-8-acetoxy-2-methylquinoline (4)



In a 500 mL round-bottomed flask containing 5,7-diamino-8-hydroxy-2-methylquinoline dihydrochloride solution, sodium acetate (30.0 g, 370 mmol) and sodium sulfite (15.0 g, 119 mmol) were added and stirred. The solution was placed in an ice-water bath, and 88 mL of acetic anhydride was added dropwise over an hour. The solution was stirred for an additional 1.5h and the yellow-white precipitate was collected via vacuum filtration to yield 11.38 g (99%).  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  2.14 (s, 3H), 2.15 (s, 3H), 2.41 (s, 3H), 2.58 (s, 3H), 7.35 (d,  $J$  = 8.6 Hz, 1H), 8.23 (d,  $J$  = 8.6 Hz, 1H), 8.31 (s, 1H), 9.76 (s, 1H), 10.02 (s, 1H)

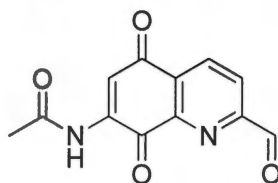
### Preparation of 7N-Acetamido-2methylquinoline-5,8-dione (5)



In a 100 mL round-bottomed flask, methanol (57 mL), water (5 mL) and 5,7N-Diacetamide-8-acetoxy-2-methylquinoline (1.0 g, 3.17 mmol) were combined and refluxed for 30 minutes. The solution was rotovapped to dryness. The dried compound was dissolved in glacial acetic acid (21 mL), and then combined with chromic acid made with potassium dichromate (2.8 g, 9.51 mmol) and water (11.3 mL). The solution was vigorously stirred for 8h. 5% Sodium bicarbonate (50 mL) was added to the dark red solution before it was extracted with

dichloromethane (70 mL, 5x25 mL). The combined organic layer was washed with 5% sodium bicarbonate (4x25mL). The yellow solution was dried with magnesium sulfate and filtered through a funnel stuffed with cotton ball. The filtrate was rotovapped to dryness to recover 0.38 g (52%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.30 (s, 3H), 2.75 (s, 3H), 7.55 (d,  $J$  = 8.0 Hz, 1H), 7.90 (s, 1H), 8.29 (d, 8.0 Hz, 1H), 8.38 (br s, 1H).

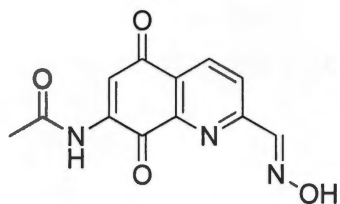
### Preparation of 7N-Acetamido-2-formylquinoline-5,8-dione (6)



Dioxane was dried and distilled prior to reaction. 7N-Acetamido-2-methylquinoline-5,8-dione (0.23 g, 1.00 mmol), selenium dioxide (0.13 g, 1.22 mmol), and dioxane (3.5 mL) were added to a 25 mL round-bottomed flask. The solution was gently refluxed under argon until the mixture became homogenous. Water (0.15 mL) was then added and the reaction ran for 16 h. Addition of minimal amount of selenium dioxide and water were used to keep solution from drying and to drive reaction to completion, which was checked by chromatography (DCM/ethyl acetate 50:50). Two glass pipettes were plugged with cotton balls and filled with celite to make a celite column. The black solution was poured into the columns and washed with 100 mL of DCM. The solution was transferred to a separatory funnel and washed with NaCl (1x15 mL) and 5% sodium bicarbonate (5x20 mL). The yellow solution was dried with magnesium sulfate and filtrated through a cotton plugged funnel. Rotovaporation of the product yielded 0.11 g (46%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.33 (s, 3H), 8.05 (s, 1H), 8.31 (d,  $J$  = 8.0 Hz, 1H), 8.43 (br s, 1H), 8.62 (d,  $J$  = 8.0 Hz, 1H), 10.29 (s, 1H)

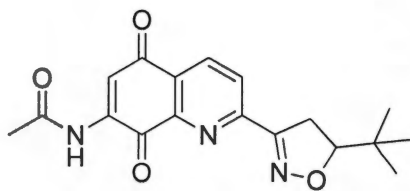


### Preparation of 7N-acetamido-5,8-dione-quinolyl-2-aldoxime (7)



In a 25 mL round-bottomed flask, 7N-Acetamido-2-formylquinoline-5,8-dione (100.0 mg, 0.41 mmol), hydroxylamine hydrochloride (31.6 mg, 0.454 mmol) and sodium acetate (54.2 mg, 0.66 mmol) were added to a solution of ethanol:water (4:1mL). The solution was stirred vigorously for 16h and then stirred in an ice-water bath for an additional 2h. Vacuum filtration of the product yielded 0.056 g (52%). <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.27 (s, 3H), 7.74 (s, 1H), 8.19 (d, J = 8.0 Hz, 2H), 8.28 (s, 1H), 8.34 (d, J = 8.1 Hz, 2H), 10.06 (s, 1H), 12.23 (s, 1H)

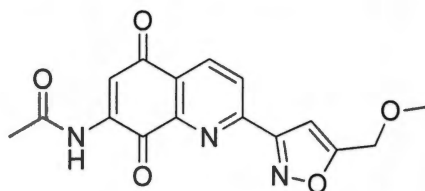
### Preparation of 7N-Acetamido-2-[5-(1,1-dimethylethyl)-4,5-dihydro-3-isoxazolyl]-quinoline-5,8-dione (8)



In a 25 mL round-bottomed flask, 7N-acetamido-5,8-dione-quinoline-2-aldoxime (0.260 g, 1.00 mmol), 3,3-dimethyl-1butene (0.422 g, 5.01 mmol) and 10 mL of DCM were combined, stirred, and then cooled to 0 °C. Bleach (sodium hypochlorite, 0.7 M, 3.0 mL, 2.1 mmol) was added dropwise over 30min. The solution was brought to room temperature and stirred vigorously for 8h. Extraction with DCM (4x10 mL) gave a yellow solution. It was dried with sodium sulfate and filtrated through a cotton plugged funnel. Rotovaporation of the product

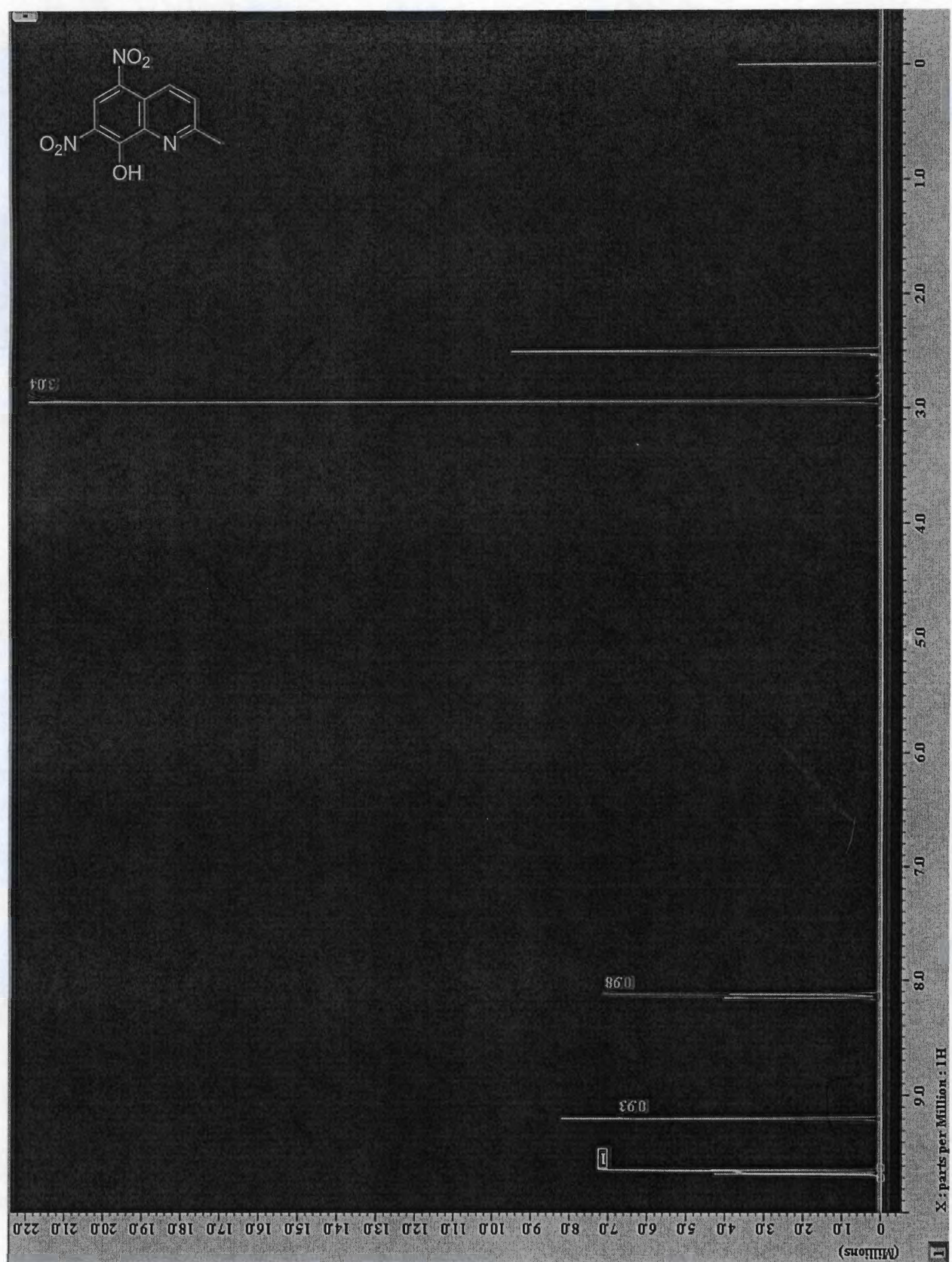
yielded 0.12 g (71%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.00 (s, 9H), 2.31 (s, 3H), 3.34 (dd,  $J = 18.0, 9.9$  Hz, 1H), 3.49 (dd,  $J = 18.0, 11.4$  Hz, 1H), 4.59 (dd,  $J = 11.4, 9.9$  Hz, 2H), 7.96 (s, 1H), 8.36 (br s, 1H), 8.38 (d,  $J = 8.4$  Hz, 1H), 8.41 (s,  $J = 8.4$  Hz, 1H)

**Preparation of 7N-acetamido-2-[5-(Methoxymethyl)-3-isoxazol]-quinoline-5,8-dione (9)**



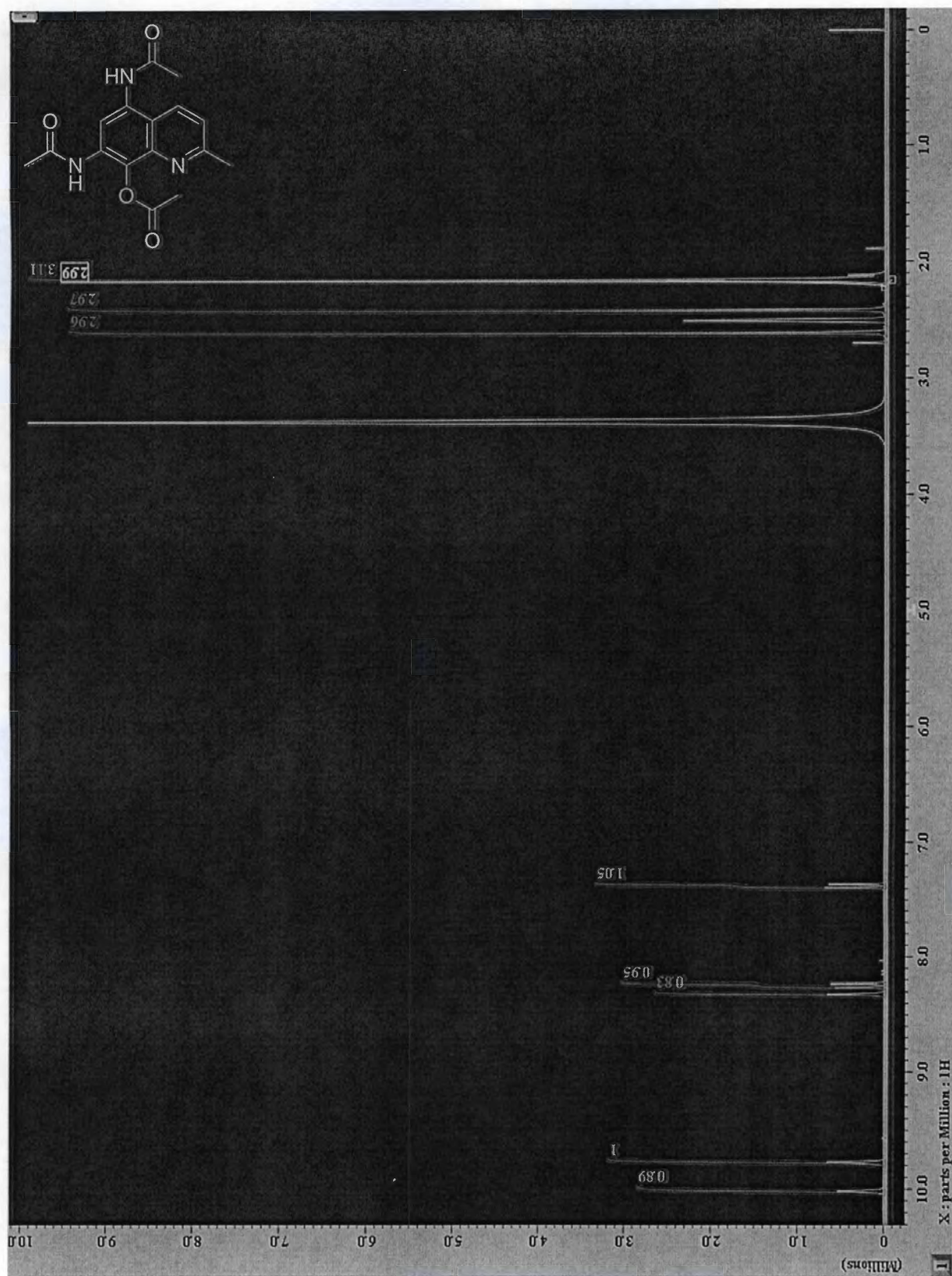
In a 50mL round-bottomed flask, 7N-acetamido-5,8-dione-quinoline-2-aldoxime (0.132 g, 0.509 mmol), methyl propargyl ether (0.303 g, 2.55 mmol) and 10mL of DCM were combined, stirred, and then cooled to 0 °C. Bleach (sodium hypochlorite, 0.7 M, 3.0 mL, 2.1 mmol) was added dropwise over 30min. The solution was brought to room temperature and stirred vigorously for 8h. Extraction with DCM (4x10mL) gave a yellow solution. It was dried with sodium sulfate and filtrated through a cotton plugged funnel. Rotovaporation of the product yielded 0.10 g (59%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.33 (s, 3H), 3.48 (s, 3H), 4.64 (s, 2H), 7.14 (s, 1H), 7.99 (s, 1H), 8.39 (br s, 1H), 8.48 (d,  $J = 8.0$  Hz, 1H), 8.54 (d,  $J = 8.0$  Hz, 1H)

<sup>1</sup>H NMR spectrum of 8-Hydroxy-2-methyl-5,7-dinitroquinoline



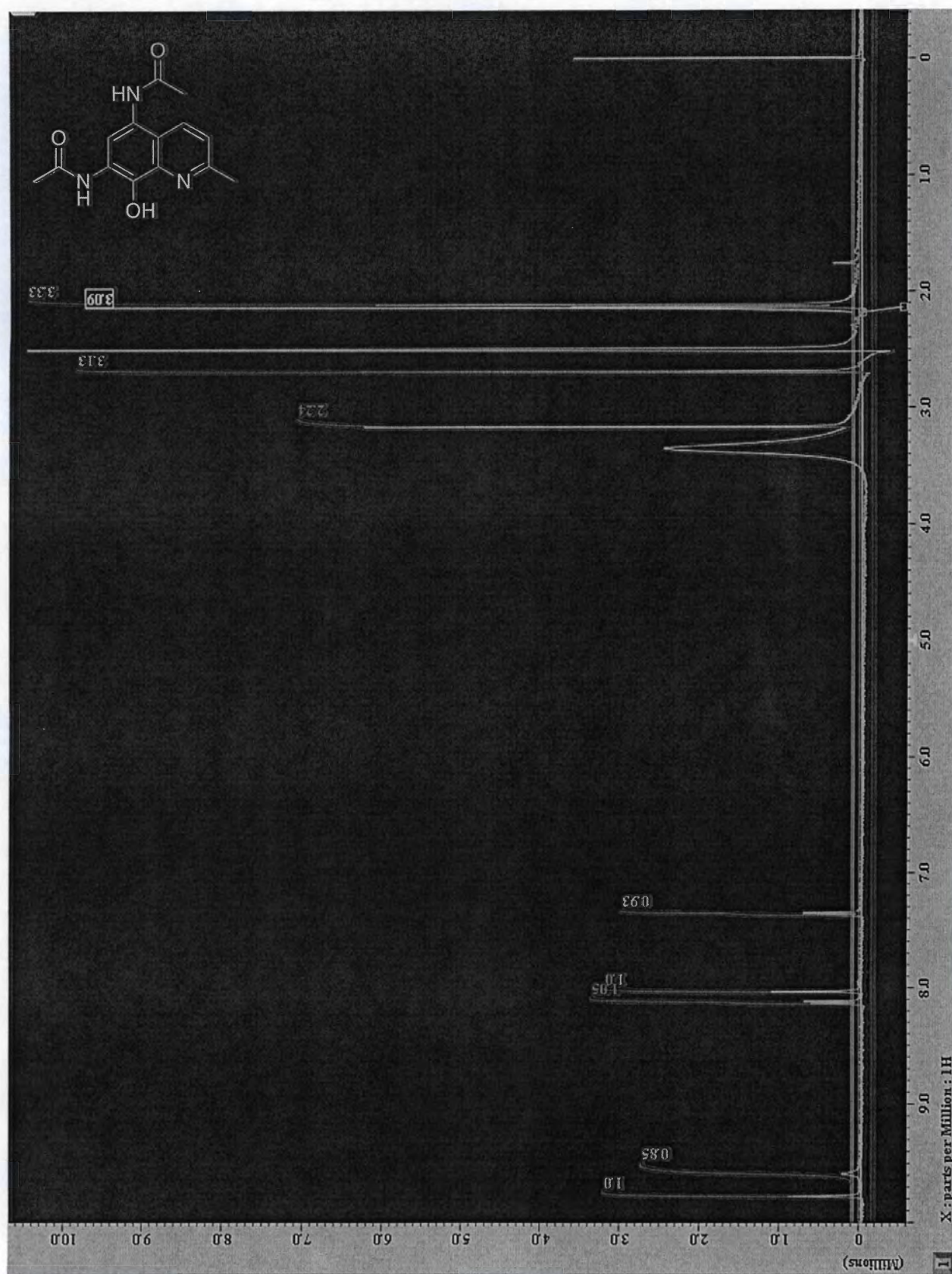


<sup>1</sup>H NMR spectrum of 5,7-Diacetamido-8-acetoxy-2-methylquinoline

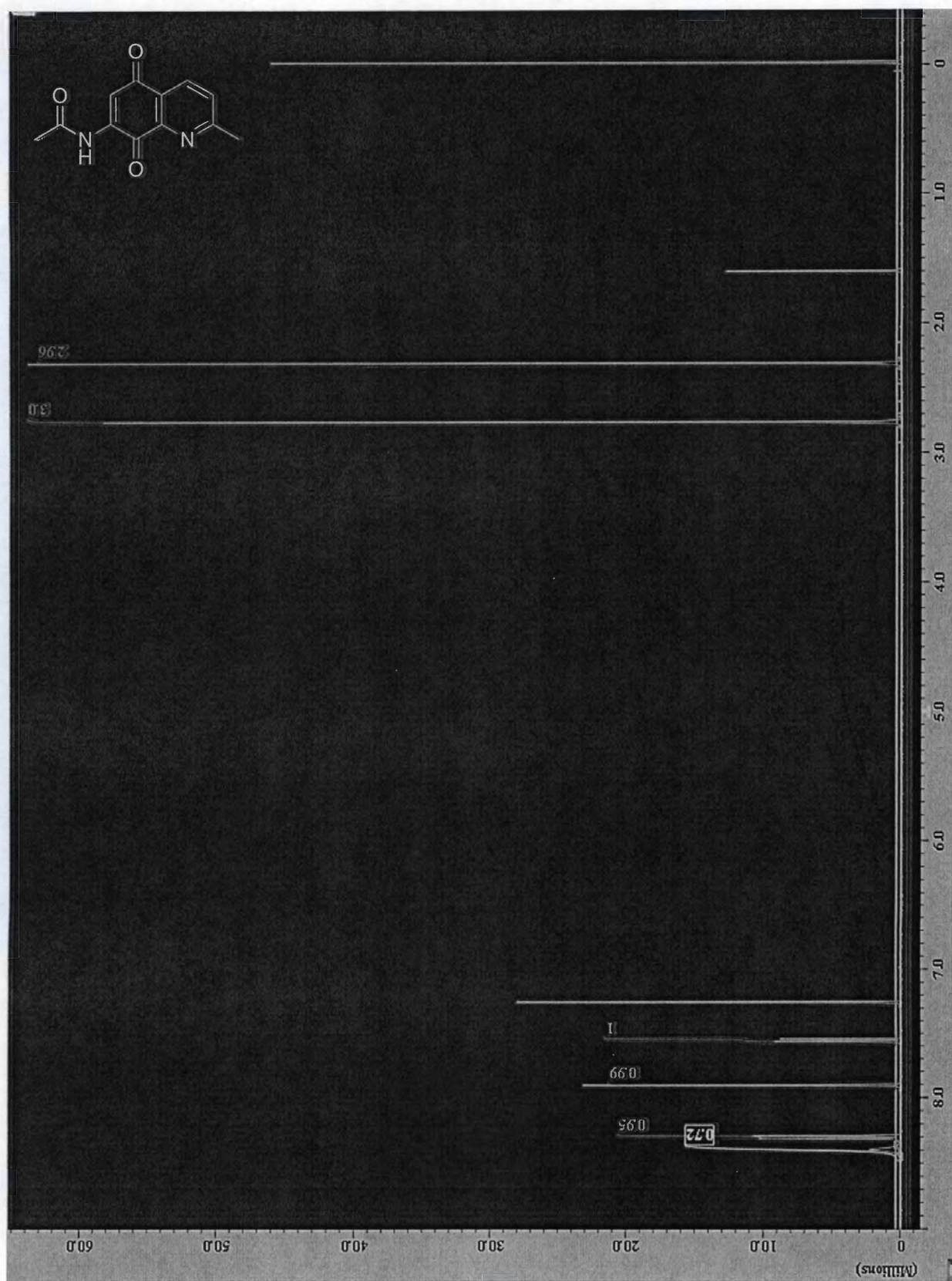




<sup>1</sup>H NMR spectrum of 5,7N-Diacetamido-8-hydroxy-2-methylquinoline

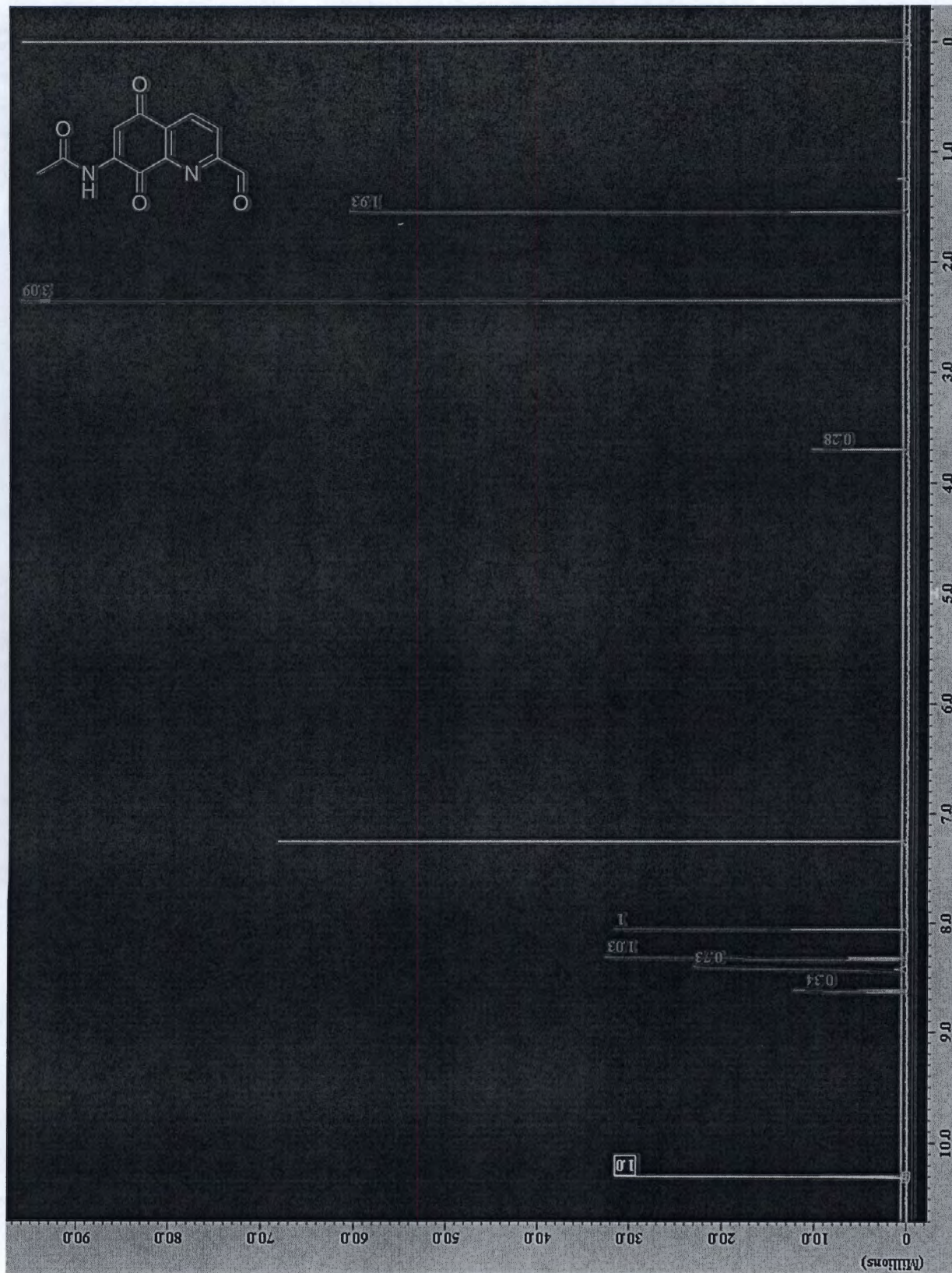


<sup>1</sup>H NMR spectrum of 7N-Acetamido-2-methylquinoline-5,8-dione



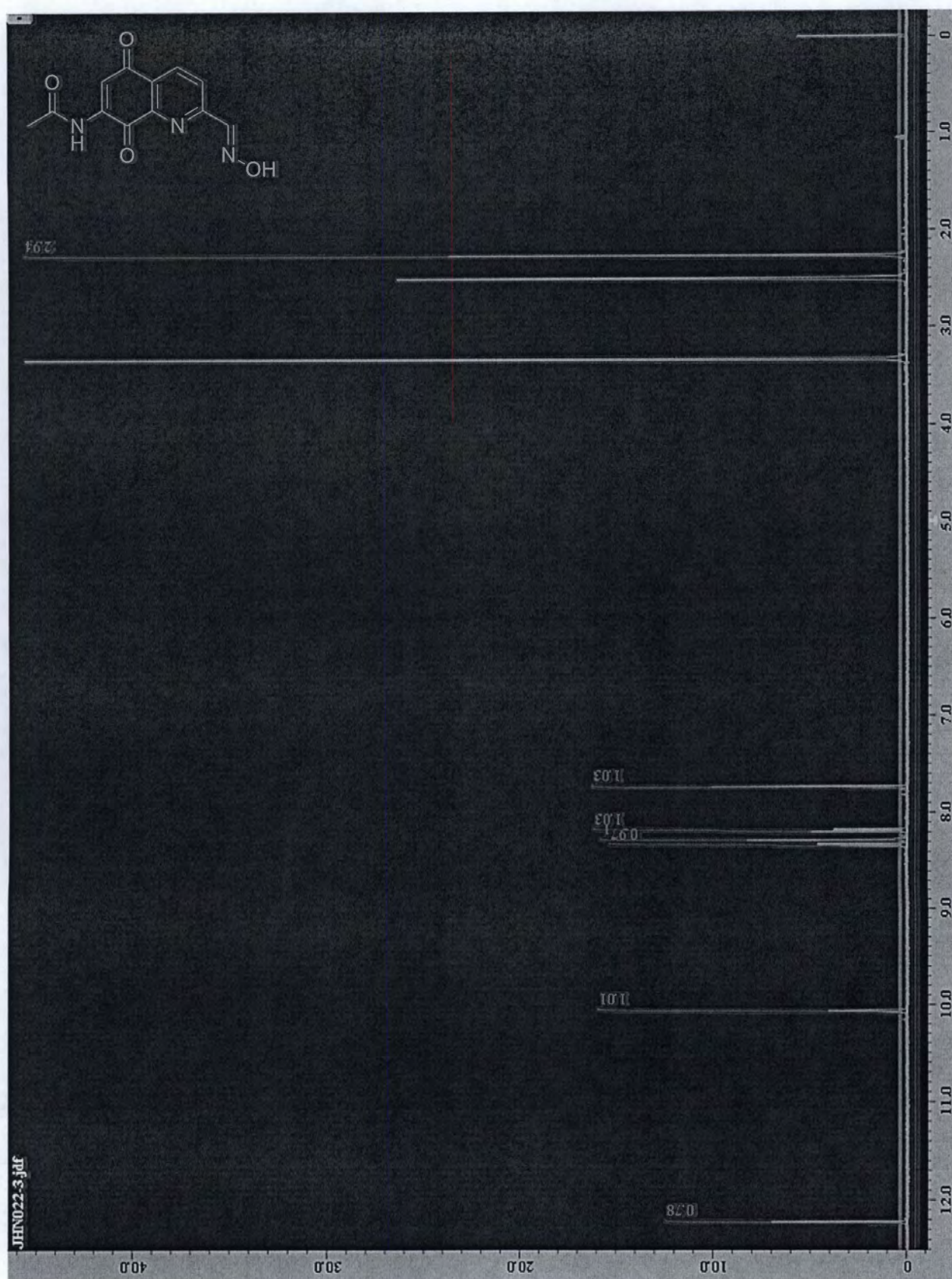


$^1\text{H}$  NMR spectrum of 7N-acetamido-2-formylquinoline-5,8-dione



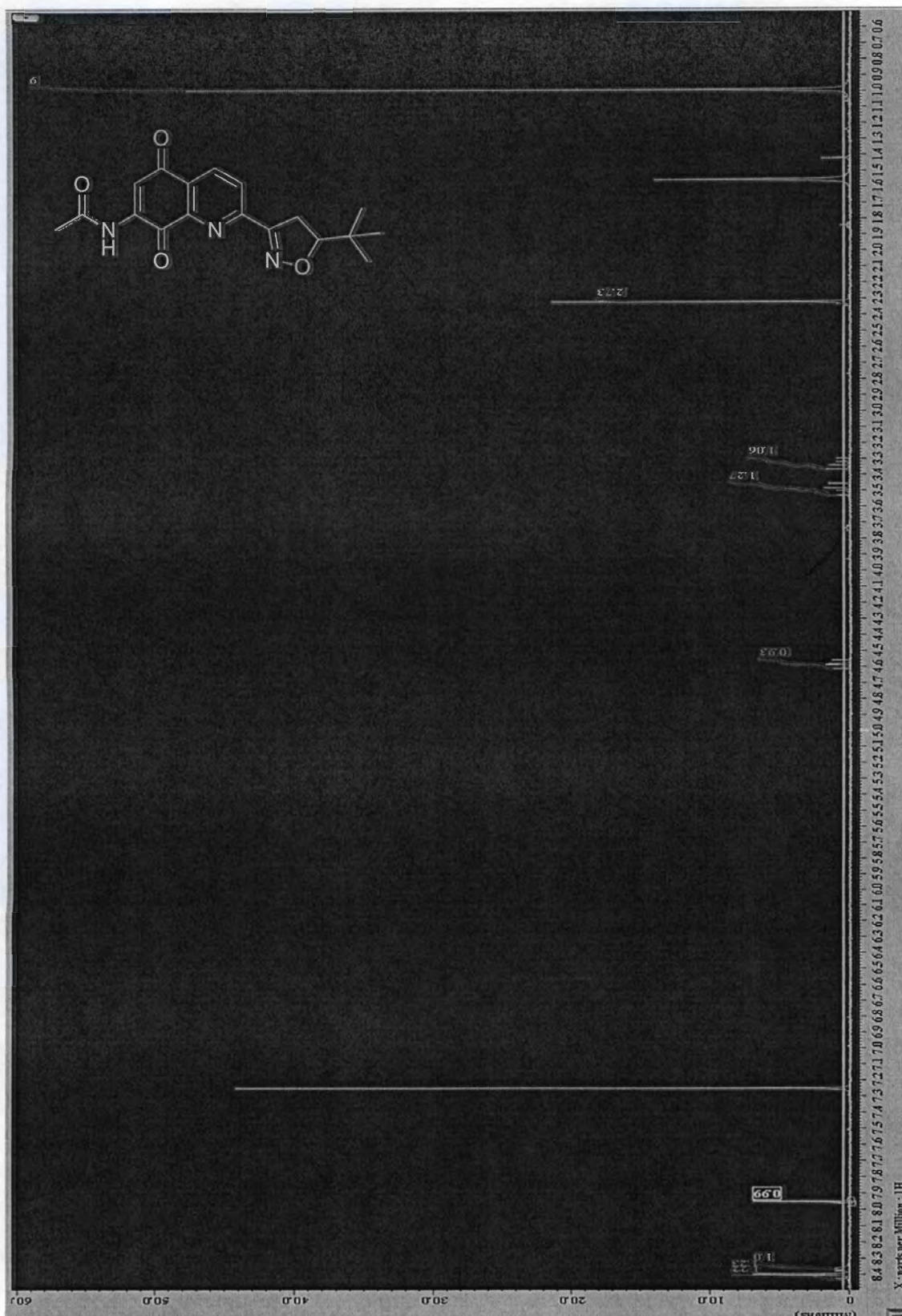


<sup>1</sup>H NMR spectrum of 7N-acetamido-5,8-dione-quinoline-2-aldoxime



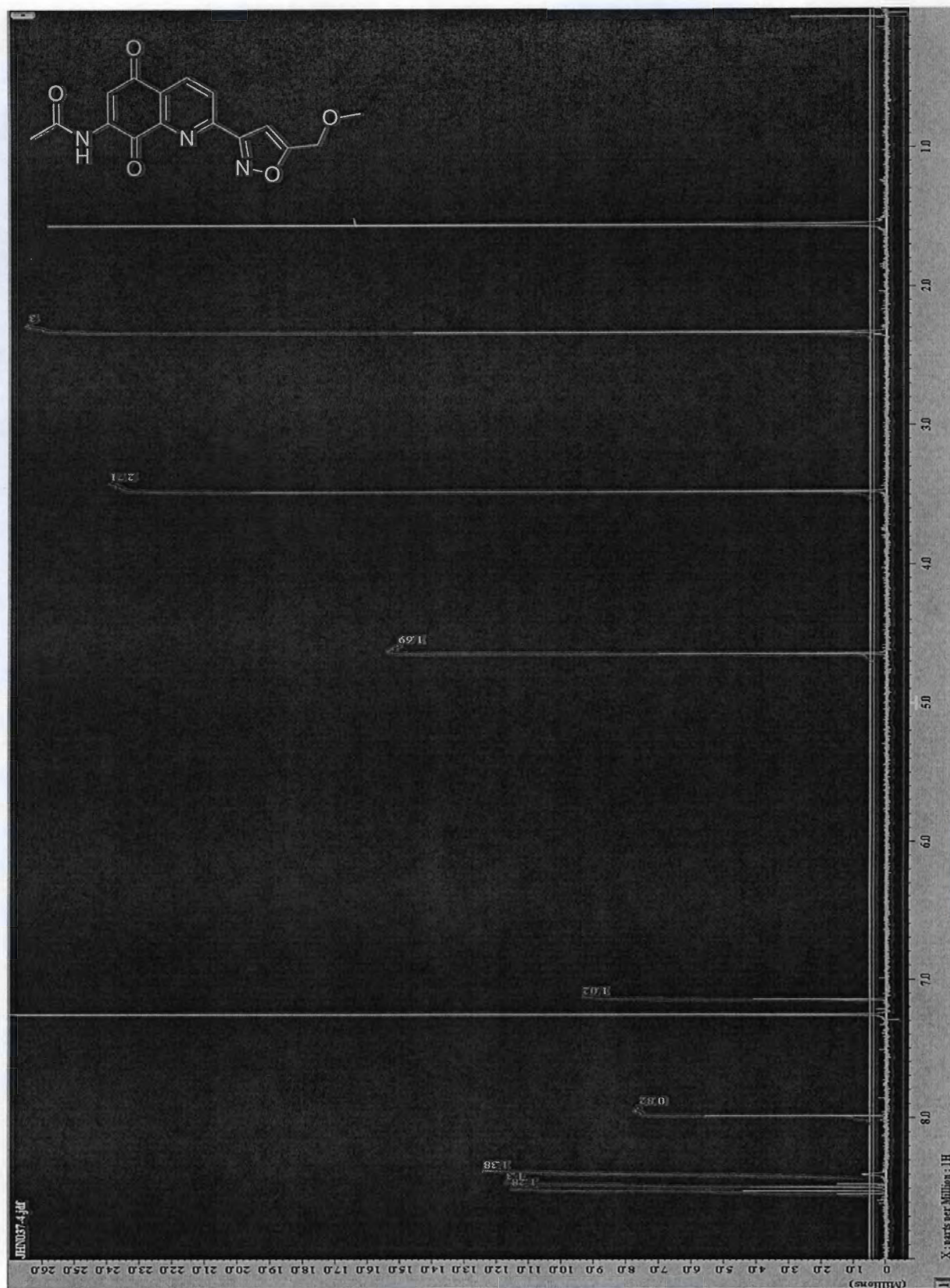


<sup>1</sup>H NMR spectrum of 7N-Acetamido-2-[5-(1,1-dimethylethyl)-4,5-dihydro-3-isoxazolyl]-quinoline-5,8-dione (8)



<sup>1</sup>H NMR spectrum of 7N-acetamido-2-[5-(Methoxymethyl)-3-isoxazol]-quinoline-5,8-dione

(9)



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